Modeling Disease Progression of Camellia Twig Blight Using a Recurrent Event Model

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ABSTRACT

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To improve control of camellia twig blight (CTB) using sanitation methods, a more complete epidemiologic understanding of this disease is necessary. Three CTB disease stages were modeled using recurrent event analysis. Wound inoculated stems were observed at regular intervals for appearance of disease symptoms. Survival times (time from inoculation until symptom appearance) for the three disease stages (mild, moderate, and severe) were regressed against stem diameter, monthly mean hours/day within a specified temperature range (15 to 30°C), and season (spring, summer, fall, and winter). For all three CTB disease stages, stem diameter had a protective effect on survival times, while monthly mean hours/day in the specified temperature range and warmer seasons were risk factors. Based upon median ratios, the mild disease stage developed 2 to 3 times faster in spring, summer, and fall than in winter. Similarly, moderate and severe disease stages developed 2 to 2.5 times faster. For all three disease stages, seasonal differences in stage development were smaller among fall, spring, and summer, varying from 1 to 1.6 times faster. Recurrent event modeling of CTB progression provides knowledge concerning developmental expression of this disease, information necessary for creating a comprehensive, integrated disease management program.

Additional keyword: pruning.

Camellia twig blight (CTB) caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. (teleomorph: *Glomerella cingulata* [Stoneman] Spauld. & H. Schrenk) is a disease common in the south central and southeastern United States. Symptoms include twig blight, leaf spot, and branch canker. The four primary disease control approaches are exclusion (exclusive planting of disease-free camellia stock produced in regions where the disease is not present), disease resistant cultivars (5), protection (use of fungicides, and site selection and maintenance to minimize conditions favorable for disease development) (4,7), and eradication (sanitation methods often involving removal of infected plant material). Camellia enthusiasts often select cultivars based on horticultural characteristics, not disease-resistant traits; hence disease-resistant cultivars are not disproportionately purchased in favor of other cultivars. Fungicides benefit control, but timing recommendations are limited to application after older leaves naturally senesce in late spring (7). Often, fungicide applications are made at the discretion of the producer, commonly in response to disease symptom build-up. Diseased twigs are removed to reduce inoculum production, but best practice guidelines that maximize sanitation impact on CTB have not been documented. Hence producers follow general horticultural pruning practices. Despite the comprehensive nature of these control methods, few research articles are available that specifically describe disease components of CTB, and few of these controls have been specifically developed or properly evaluated for CTB.

The nature of disease control in ornamental plants generally differs from traditional agricultural crops. For most crops, disease control is scheduled around crop phenology with harvesting high yields of a quality fiber or food product being the primary objective. For ornamentals, the primary objective is overall plant aesthetics throughout the year; thus the association with crop phenology is often less specific and useful.

Basic disease control methods strive to reduce initial disease intensity and/or slow the rate of disease development, regardless of the cropping system. Typically, identifying the occurrence of inoculum production periods, inoculum dispersal events, and susceptibility of plant stages are primary disease components considered in disease control method (chemical, cultural, and sanitation) selection and application timing. As a pathogen, *C. gloeosporioides* can produce conidia from February to November in tropical and semitropical climates (11,31). In theory then, CTB infection is possible throughout much of the year. This possibility contradicts the literature which states that the primary infection event occurs during several weeks in the spring when older leaves naturally senesce (7). One approach that can be used to test this theory is to determine the seasonal time from infection to symptom development in all seasons of the year. A seasonal incubation period has previously been described and calculated (8). We wanted to expand that information to determine the seasonal pattern from infection to mild (initial, incubation period), moderate, and severe (most advanced) symptoms. While the utility of this approach is untested, we hypothesize that a reverse-deductive approach can be used to determine when an infection may have occurred based upon symptom appearance.

Because disease progression involves time to event data, survival analysis is an ideal tool to model these data. Most survival analyses involve a single event; however, it is not uncommon for multiple events to occur for a single experimental unit. Modeling multiple (or recurrent) events involves the use of multivariate survival techniques which take into account the structure between events. While a number of proportional hazards-type models have been proposed for use with recurrent event data, the choice of

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which model to use is highly dependent on the interpretation of model coefficients. Model choice should be based upon the goals of the analysis such that the interpretation of the model coefficients is appropriate to the research question (15).

Survival analysis has not been commonly used in the natural sciences such as botany and plant pathology; however, recent articles provide evidence for its rise in popularity (3,9,10,22, 23,30,32). Scherm and Ojiambo (25) provide a more complete discussion on the use of survival analysis in botanical applications. However, very few (if any) of these studies involve the use of survival analysis with recurrent event data.

The purpose of this study was to model the length of time from infection through multiple stages of CTB disease symptoms using a marginal recurrent event model. Modeling CTB symptom progression will give a more complete epidemiologic picture of this disease. Understanding seasonal symptom appearance and development could provide insight into timing of chemical and sanitation methods for the most efficacious control of this disease.

MATERIALS AND METHODS

Data collection. Two-year-old *Camellia sasanqua* 'Rosea' plants were inoculated with *C. gloeosporioides* over the course of a 2-year period. A total of 274 camellia stems were inoculated in 22 different periods over the course of 23 months, spanning three calendar years. For year one (July 2003 to June 2004), inoculation periods corresponded to all calendar months except for the month of January. For year two (July 2004 to May 2005), inoculation periods corresponded to all calendar months except for the month of June. On average, three plants were inoculated in each period. The minimum and maximum number of stems inoculated per period was 10 and 17, respectively. Details regarding pathogen inoculation and subsequent treatment have been outlined elsewhere (8). In brief, sets of camellia plants were wound inoculated with a virulent isolate of *C. gloeosporioides* (CG02g) at monthly intervals. The single isolate was used throughout the study to minimize variability. After 3 days in the greenhouse, plants were placed outdoors and monitored for symptoms of disease at 2- to 3-day intervals. Daily temperature was recorded in 15-min intervals.

Three progressive stages of disease symptoms (mild, moderate, and severe) were observed distal of the inoculation point. The first stage of disease (called mild) was characterized by chlorotic, reddish, or wilted dull green-gray colored leaves, or leaves with necrotic margins. The second and third stages of disease (called moderate and severe, respectively) were characterized by at least one completely necrotic leaf and by complete necrosis of all leaves, respectively. Periods were terminated when all stems had become completely blighted or no changes occurred in a few remaining stems for two or more weeks, thus resulting in periods non-uniform in length.

Assumption of independence. Most statistical methods rely on the assumption of independence of the observations. Violation of this assumption was a potential concern for this study since multiple twigs from the same plants were included in the analyses. To ensure that correlation among twigs of the same plants was not a factor which needed to be accounted for in the analyses, spatial autocorrelation techniques (Moran's *I* and Geary's *c*) were applied. Moran's *I* statistic is computed by standardizing the spatial autocovariance by the variance of the data (21). Moran's *I* values range from –1 to 1 with values greater than 0 representing positive autocorrelation, values less than 0 representing negative autocorrelation, and values close to zero indicating no autocorrelation. Geary's *c* statistic is computed by summing the squared differences between pairs of data values (12). Geary's *c* values range from 0 to 2 with values close to 0 representing strong positive autocorrelation, values close to 2 representing strong negative autocorrelation, and values close to 1 indicating no autocorrelation. For both methods, the variable of interest is time to symptom appearance, the spatial variable is plant, and pairs of data values are twigs on the same plant versus twigs on different plants. Because of the small number of twigs used per plant, *P* values (*P*) were computed based on randomization tests (versus relying on the normal approximation). In the presence of significant autocorrelation among twigs of the same plant, additional analytic measures would need to be used (15).

Recurrent event model. The time (in days) from inoculation until occurrence of the three disease stages was modeled using a recurrent event model for survival analysis. Specifically, a stratified extended Cox model using the exact procedure for handling ties (assumes ties are the result of imprecise measuring) and the fixed effects partial likelihood (FEPL) method was chosen to model the data. The FEPL method allows for a separate baseline hazard function for each disease stage and corrects for some or all of the bias in the model coefficients caused by unobserved heterogeneity (2).

A marginal model was chosen since this is the suggested approach when recurrent events are viewed to be of different types and the order of events is important (17). In the marginal approach, each twig is considered to be at risk for all disease stages, regardless of the number of events that were actually observed for the twig. Hence, the start times for all three disease stages were the same - the beginning of the inoculation period. If all three disease stages were observed, then the end times were the respective days on which the stages were observed. For cases in which an earlier stage (mild or moderate) was not observed, but a subsequent stage (moderate or severe) was observed, the end times were assigned as the day on which the subsequent stage was observed. For example, if the only stage observed was the severe stage, then end time for all three stages was the day on which the severe stage was observed. In other words, the stages were assumed to have occurred concurrently with no gap in the appearance of all three stages. In this manner, while a disease stage may not have been recorded, if a subsequent stage was observed, it was assumed that the preceding stage did occur, but was simply not observed. While it can be argued that the preceding stage may not have occurred, we chose to model the data in this fashion for two reasons. First, the development of twig blight is a progressive, sequential process characterized by colonization of internal twig tissues that eventually becomes sufficiently extensive to cause physiological stress and death of all leaf and stem tissues distal of the infection site. While visual distinctive symptoms were successive in order, the stages are somewhat arbitrary since the rate of colonization and other factors affect the nature and development of symptoms. Second, we wished to avoid the illogical situation in which survival time for an earlier stage extended beyond that of a later stage. If none of the three disease stages were observed, stems were defined as censored for all three stages.

The stratified extended Cox hazard regression function has the following form: $h_s(t,x,\beta_s) = h_{0s}(t)\exp[x'(t)\beta_s]$, where t is the time to event, $\boldsymbol{x}$ is a vector of covariates or explanatory variables some of which may depend on time, β_s is a vector of unknown parameters to be estimated for the sth event (s = 1, 2, and 3), $h_{0s}(t)$ is a baseline hazard function that involves time but not the explanatory variables for the sth event, and $\exp[x'(t)\beta_s]$ is a linear function of a set of explanatory variables for the sth event that accounts for time dependence such as nonproportional hazards in the model.

Robust sandwich estimator. An assumption of Cox regression models is that all events are independent of one another. Since this is not true for recurrent event data (i.e., observations on the same unit may be correlated), adjustments must be made to account for the correlation among experimental units. To account for misspecification of the correlation structure assumed, estimated variances of the regression coefficients obtained for a fitted model are adjusted (16,33). This adjustment is accomplished

using the robust sandwich estimate of the covariance matrix (18) which allows for dependence among multiple event times.

The robust sandwich estimator for recurrent events has the following form written in matrix notation: $R(\beta) = \mathrm{Var}_{est}(\beta)[R_s'R_s]\mathrm{Var}_{est}(\beta)$, where $\mathrm{Var}_{est}(\beta)$ is the information matrix form of estimated variances and covariances obtained from partial maximum likelihood estimation of the Cox model being fit, and R_s is a matrix of score residuals obtained from the maximum likelihood estimation.

Explanatory variables. The selection of the explanatory variables included in the models was based upon biological and empirical reasons which will not be discussed here but have been described elsewhere (8). The set of explanatory variables included stem diameter, monthly mean hours/day within the temperature range of 15 to 30°C for the month following inoculation, season, and season by time interaction to account for possible nonproportionality in the season variable. The categorical season variable was composed of four classes representing different growth phases associated with the four seasons of the year, and based on a combination of observational notes and leaf-growth data taken from the plants. The winter season representing dormancy was composed of December, January, February, and March. The spring season representing leaf and stem growth was composed of April, May, and June. The summer season representing stem hardening and bud set was composed of July, August, and September. The fall season representing cessation of leaf and stem growth and opening of flowers was composed of October and November. Since each of the three disease stages was treated as a separate stratum for analysis, stratum-specific variables were created for the explanatory variables.

All statistical analyses were performed using SAS software, version 9.1 (SAS Institute Inc., Cary, NC). Results were considered significant at the 0.05 nominal level.

RESULTS

For each of the four seasons, summary data for the number of periods, number of stems inoculated, number of uncensored stems (stems which developed symptoms), and number and percentage of stems in each CTB disease stage pattern are reported in Table 1. The number of periods in each season ranged from 4 (fall) to 7 (winter). The number of inoculated stems in each season ranged from 48 stems (fall) to 90 stems (winter).

In uncensored stems, all four patterns of disease stage occurrence were observed in each of the four seasons (Table 1). The most prevalent pattern ($1 < 2 = 3$), accounting for 49.8% of uncensored stems, was defined by a distinct occurrence of the mild stage followed by occurrence of the severe stage. The moderate stage, while assumed to have occurred, was not observed. This was the most prevalent pattern for spring and fall. The second most prevalent pattern ($1 < 2 < 3$) accounted for 37.0% of uncensored stems. This pattern was defined by the distinct sequential occurrence of the mild, moderate, and severe stages. This was the most prevalent pattern for summer. For winter, the majority of stems was equally split between the two patterns, $1 < 2 = 3$ and $1 < 2 < 3$. The second least prevalent pattern ($1 = 2 = 3$), accounting for 9.6% of uncensored stems, was defined by a distinct occurrence of only the severe stage. The occurrences of the mild and moderate stages were not observed. The least prevalent pattern ($1 = 2 < 3$), accounting for 3.6% of uncensored stems, was defined by the distinct occurrences of the moderate and severe stages. The occurrence of the mild stage was not observed, although it was assumed to have occurred.

Assumption of independence. Results from the spatial autocorrelation were similar for Moran's *I* and Geary's *c* in all cases except one (Moran's *I* indicated significance while Geary's *c* did not). Hence, test statistics and *P* values (*P*) are reported in Table 2 for Moran's *I* only. For the mild disease stage, periods represented by March (first year) and May (first and second years) were found to have significant autocorrelation ($P = 0.0030$, 0.0027 and 0.0091, respectively). For the moderate and severe disease stages, periods represented by March and April of the first year were found to have significant autocorrelation ($P = 0.0041$, 0.0044, 0.0031, and 0.0044, respectively). Because so few periods had significant autocorrelation present among twigs of the same plant, the data were treated as independent observations. The few periods found to have significant autocorrelation may truly represent dependence among twigs. Alternatively, they could have resulted from inflated Type I error rates which can occur when using unadjusted rates for multiple testing.

Explanatory variables. Explanatory variables used in the recurrent event model included stem diameter, a continuous variable representing monthly mean hours/day within the temperature range of 15 to 30°C for the month following inoculation, a continuous season variable, and an interaction term for season and time to account for the nonproportionality present in the season variable. Although the season variable is categorical in nature (spring, summer, fall, and winter), the decision to model it on a continuous scale was based upon parsimony (3 stratum [symptom categories] × 4 seasons = 12 additional variables in the model) and the interpretive value of the hazard ratios. Numeric values based upon descending median survival length were assigned to the seasons as follows: winter (1, representing the longest median survival time), fall (2), summer (3), and spring (4, representing the shortest median survival time).

Results based on median survival times indicate that spring had the shortest survival times followed by summer, then fall, while winter exhibited the longest survival times (Table 3). For the winter season, median survival times for the mild, moderate, and severe disease stages were 58.0, 62.5, and 65.0 days, respectively. For the fall season, median survival times for the three stages were 29.0, 34.0, and 36.5 days, respectively. For the summer season, median survival times for the three stages were 23.0, 25.5, and 27.5 days, respectively. For the spring season, median survival times for the three stages were 18.0, 25.0, and 27.0 days, respectively.

Following the advice of Spruance et al. (27), median ratios were computed in a pair-wise fashion for the seasons (Table 4).

TABLE 1. Patterns of camellia twig blight disease stage occurrence in uncensored stems

Season	No. of periods	No. of uncensored/total stems	Patterns of stage occurrence in uncensored stems [n(%)]			
			$1 < 2 < 3$[a]	$1 < 2 = 3$[b]	$1 = 2 < 3$[c]	$1 = 2 = 3$[d]
Spring	5	76/78	24 (31.6)	48 (63.2)	1 (1.3)	3 (4.0)
Summer	6	49/58	23 (46.9)	18 (36.7)	1 (2.0)	7 (14.3)
Fall	4	45/48	10 (22.2)	23 (51.1)	5 (11.1)	7 (15.6)
Winter	7	79/90	35 (44.3)	35 (44.3)	2 (2.5)	7 (8.9)
Total	22	249/274	92 (37.0)	124 (49.8)	9 (3.6)	24 (9.6)

[a] Sequential appearance of all three disease stages (1 = mild, 2 = moderate, 3 = severe).
[b] Sequential appearance of mild and severe disease stages; moderate stage unobserved.
[c] Sequential appearance of moderate and severe disease stages; mild stage unobserved.
[d] Only severe disease stage observed.

Median ratios are a measure of rate, providing an estimate of the magnitude of benefit in CTB disease progression. For the mild disease stage, infected stems exposed to temperatures during spring, summer, and fall developed symptoms at 3.2, 2.5, and 2.0 times faster rates, respectively, than infected stems exposed to winter months. For the moderate and severe stages, these rates were 2.5, 2.5, and 1.8; and 2.4, 2.4, and 1.8 times faster, respectively.

Recurrent event model. Stem diameter had estimated hazard ratios of 0.55, 0.62, and 0.61 for the mild, moderate, and severe stages, respectively (Table 5). These hazard ratios indicate a consistent protective effect of stem diameter on occurrence for all three stages. Every 1 mm increase in stem diameter resulted in 45, 38, and 39% decreases in risk of mild, moderate, and severe occurrences, respectively. Monthly mean hours/day within the temperature range of 15 to 30°C for the month following inoculation had estimated hazard ratios of 1.10 for the mild, moderate and severe stages indicating negative effects (Table 5). Every 1 h increase in mean hours/day within the temperature range of 15 to 30°C resulted in 10% increases in risk of mild, moderate, and severe occurrences. Season had estimated hazard ratios of 2.68, 2.63, and 2.60 for the mild, moderate, and severe stages, respectively (Table 5). This negative effect of season indicated that on average, moving from one season to another (e.g., winter to fall, fall to summer, summer to spring) resulted in almost three-fold increases in risk for mild, moderate, and severe occurrences. Because the hazard ratios for the disease stages' interaction terms were less than one, the seasonal hazard ratios were decreasing over time. Thus the greatest differences in risk of disease stage occurred between the winter and fall seasons (versus the fall and summer, and summer and spring comparisons).

The relationship between the three disease stages in terms of hazard functions is illustrated in Figure 1. All three curves appear quite similar in shape with the hazard functions increasing in approximate linear fashion over time. The mild stage hazard curve indicated that risk was essentially zero for the first 11 days of infection, whereas this zero-risk period extended out to approximately days 16 and 17 for the moderate and severe stages, respectively. The mild stage curve was always above those of the

TABLE 2. Results of spatial autocorrelation analysis using Moran's *I* for testing dependence among twigs of the same camellia plant

	Mild stage		Moderate stage		Severe stage	
Period by month and year[a]	Moran's *I*	*P*	Moran's *I*	*P*	Moran's *I*	*P*
July 2003	–0.03	0.4199	–0.04	0.4316	0.00	0.3988
August 2003	–0.24	0.2326	–0.28	0.1822	–0.32	0.1443
September 2003	0.04	0.2316	0.02	0.2814	–0.16	0.3702
October 2003	–0.08	0.4789	–0.11	0.4653	–0.12	0.4418
November 2003	0.02	0.3090	–0.06	0.4395	0.16	0.1286
December 2003	–0.07	0.4663	–0.04	0.4419	–0.07	0.4589
February 2004	–0.17	0.3108	–0.12	0.4217	–0.04	0.3583
March 2004	0.44	0.0030[b]	0.42	0.0041[b]	0.44	0.0031[b]
April 2004	0.20	0.0783	0.46	0.0044[b]	0.46	0.0044[b]
May 2004	0.32	0.0027[b]	–0.21	0.2217	–0.17	0.2921
June 2004	–0.12	0.3921	–0.20	0.2400	–0.27	0.1312
July 2004	–0.06	0.4452	–0.06	0.4357	–0.06	0.4348
August 2004	0.16	0.1145	0.13	0.1325	0.19	0.0698
September 2004	0.03	0.2645	–0.13	0.4243	–0.19	0.3206
October 2004	0.23	0.0625	0.03	0.2743	0.10	0.1865
November 2004	0.13	0.1162	0.10	0.1723	0.12	0.1539
December 2004	0.24	0.0627	–0.19	0.3190	–0.20	0.2980
January 2005	–0.33	0.1152	–0.20	0.2920	–0.25	0.2269
February 2005	–0.11	0.4411	–0.03	0.3400	–0.05	0.4146
March 2005	–0.30	0.1427	–0.31	0.1406	–0.27	0.1923
April 2005	–0.23	0.2284	–0.31	0.1394	–0.30	0.1575
May 2005	0.36	0.0091[b]	–0.19	0.2691	–0.32	0.0905

[a] Month following inoculation for respective period.
[b] *P* value significant at 0.05 level.

TABLE 3. Descriptive statistics for camellia twig blight disease stages

Season	Number of stems	Censor[a] [n(%)]	Median survival time (days)	95% CI[b]
Mild disease stage				
Spring	78	2 (2.6)	18.0	17.0, 21.0
Summer	58	9 (15.5)	23.0	20.0, 24.0
Fall	48	3 (6.3)	29.0	28.0, 37.0
Winter	90	11 (12.2)	58.0	42.0, 63.0
Total	274	25 (9.1)		
Moderate disease stage				
Spring	78	2 (2.6)	25.0	22.0, 31.0
Summer	58	9 (15.5)	25.5	25.0, 27.0
Fall	48	6 (12.5)	34.0	30.0, 43.0
Winter	90	13 (14.4)	62.5	52.0, 73.0
Total	274	30 (11.0)		
Severe disease stage				
Spring	78	2 (2.6)	27.0	24.0, 33.0
Summer	58	9 (15.5)	27.5	26.0, 30.0
Fall	48	6 (12.5)	36.5	30.0, 43.0
Winter	90	13 (14.4)	65.0	60.0, 76.0
Total	274	30 (11.0)		

[a] Number and percent of stems that did not exhibit disease stage symptoms during study period.
[b] 95% confidence interval (CI) of median survival time.

moderate and severe stages, except during the interval of days 100 to 125 in which the mild and severe stage curves crossed one another several times. The moderate stage curve was above the severe stage until approximately day 90 at which the severe stage curve exceeded the moderate stage curve. Until approximately day 84, the moderate and severe stage curves were closer to one another than either curve was to the mild stage curve. However, after day 84, the mild and severe stage curves were closer to one another than either was to the moderate stage curve.

DISCUSSION

Survival analysis using a recurrent event model provides insight into the length of time to progress from infection through increasingly severe stages of CTB symptoms. In this study, the progression of CTB had four distinct patterns whose prominence varied by season. Diseased plants were more likely to express the full range of sequential symptoms (mild-moderate-severe) in the summer season, more likely to rapidly progress from the mild to severe stage during spring and fall seasons, and equally likely to express these two patterns (mild-moderate-severe and mild-severe) in the winter season. Symptom recognition is a necessary part of disease detection and important for implementing both plant care and disease control practices. While the incubation period is of interest to plant pathologists, symptoms appearing in the later stages are more likely to be recognized by commercial plant producers and lay individuals, and thus more relevant to such individuals. It is interesting that the mild disease stage was rarely unobserved in these patterns of symptom progression. If commercial plant producers and lay individuals could be trained to recognize early symptoms of CTB, sanitation methods such as blighted twig removal may prove more efficacious than when practiced in later stages of the disease.

Temporal components of CTB that are important factors to understanding disease development include infection, incubation period (time from infection to appearance of a symptom), latent period (time from infection to production of infectious units), and tissue removal (cessation of infectious unit production). The use of seasonal inoculum production patterns, plant susceptibility stages, and progression of symptom appearance within seasons will provide a basis to predict timing of control applications. Chemical controls are applied to prevent infection, while sanitation controls are performed to remove plant tissue before inoculum is produced (without causing subsequent increased susceptibility to infection through wounding or promotion of new growth). The efficacy of these control approaches is affected by the discreteness and singleness of infection events. With many polycyclic diseases, control becomes more difficult when inoculum production events occur in closely spaced intervals over an extended period of time during which plant tissue is susceptible.

At this time, limited knowledge is available concerning the seasonal inoculum production periods and plant susceptibility stages for CTB. It is known that infection occurs through fresh leaf scars as older leaves senesce in the spring. The inoculation method using cut leaf petioles in this study simulates a similar infection route and similar stem diameter to infection at the point where older leaves naturally senesce. While leaf spots generally

TABLE 4. Median ratios for comparing camellia twig blight disease stages in different seasons

	Median ratio		
Comparison	Mild	Moderate	Severe
Winter/spring	3.2	2.5	2.4
Winter/summer	2.5	2.5	2.4
Winter/fall	2.0	1.8	1.8
Fall/spring	1.6	1.4	1.4
Fall/summer	1.3	1.3	1.3
Summer/spring	1.3	1.0	1.0

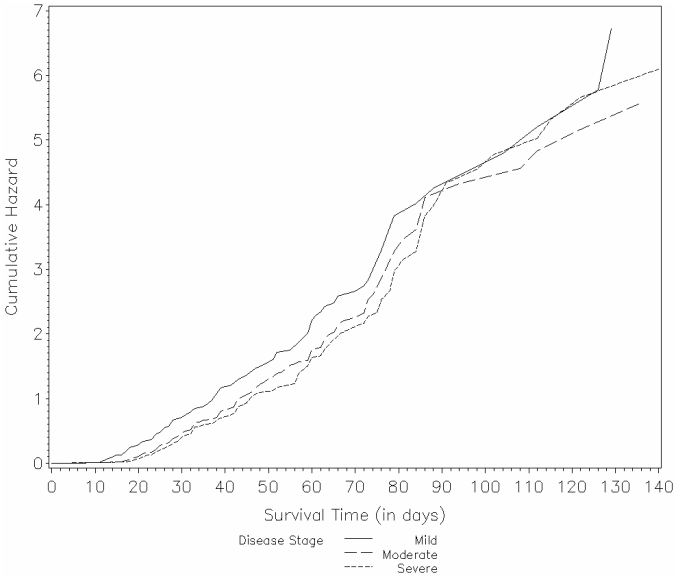

Fig. 1. Cumulative hazard function curves for the three camellia twig blight disease stages (mild, moderate, and severe) using a marginal data structure.

TABLE 5. Results of the recurrent event marginal model analysis of camellia twig blight disease progression

Explanatory variable	Hazard ratio	SE[a]	95% CI[b]	*P*
Mild disease stage				
Stem diameter	0.548	0.1279	0.426, 0.704	<0.0001
Monthly mean hours/day in temperature range[c]	1.101	0.0181	1.063, 1.141	<0.0001
Season	2.682	0.1449	2.019, 3.562	<0.0001
Season × time interaction[d]	0.983	0.0044	0.975, 0.992	0.0001
Moderate disease stage				
Stem diameter	0.620	0.1208	0.489, 0.786	<0.0001
Monthly mean hours/day in temperature range[c]	1.097	0.0170	1.061, 1.134	<0.0001
Season	2.632	0.1625	1.914, 3.619	<0.0001
Season × time interaction[d]	0.987	0.0043	0.978, 0.995	0.0018
Severe disease stage				
Stem diameter	0.613	0.1182	0.486, 0.773	<0.0001
Monthly mean hours/day in temperature range[c]	1.099	0.0167	1.064, 1.136	<0.0001
Season	2.598	0.1597	1.900, 3.553	<0.0001
Season × time interaction[d]	0.989	0.0040	0.981, 0.996	0.0037

[a] Standard error (SE) of model coefficient.
[b] 95% confidence interval (CI) of hazard ratio.
[c] Postinoculation month mean hours/day within temperature range of 15 to 30°C for 22 periods (inoculation months).
[d] Interaction accounts for nonproportionality present in the season variable.

develop in the summer, it is not known if leaves are infected from inoculum present during the leaf senesce period or from inoculum produced from twigs blighted following leaf senesce (4,7). Even fewer details are known about twig blights that develop in the summer and fall, or about infection of flower buds in the late fall.

C. gloeosporioides can produce conidia from February to November on other woody perennial crops in semi-tropical (mango) and tropical (coffee) climates (11,31). Camellias are grown in moderate to milder temperate and semitropical zones in the United States (zones 7 to 10). Thus conidia could be produced throughout much of the year under the warmer temperate climatic conditions in the southern United States. Although we lack knowledge of inoculum production periods and plant susceptibility stages for CTB, the time from infection to multiple twig blight stages could be useful in control studies that evaluate the efficacy of multiple and/or alternative timings of fungicide applications, and/or pruning if further reduction in disease intensity is needed.

Twig blight studies of various diseases have used pruning as a sanitation method during every season from spring to winter. This would imply that either the logic behind choosing the optimal time for inoculum reduction varies considerably among pathogens, or insufficient knowledge exists to fully understand the impact of seasonal pruning. Removal of blighted twigs in spring resulted in 70% reduction in infection by *Leucostoma* spp. of peach (6), 20% reduction of Cytospora canker of peach (19), and no reduction in dogwood anthracnose (20). Removal of blighted twigs in summer resulted in 17% reduction in blossom blight of sour cherry (13), and no reduction in Nectria canker of apple (24). Removal in spring and fall resulted in a 44% average reduction in Phomopsis twig blight in peach trees (28). Removal of blighted twigs in winter resulted in 79% reduction in Botryosphaeria blight of pistachio (14). Shtienberg et al. (26) found that the highest reduction of fire blight in pear occurred with December pruning compared to May, June, or August pruning. Based upon the variability of these studies' reported results, it is difficult to assess which factors may have been most influential on blight disease reduction.

Selection of a single optimum time for twig blight removal may rely on several characteristics of the pathogen and disease development. It is not known whether a single yearly pruning is more efficacious than multiple prunings spaced throughout the year. Only studies concerning fire blight on pear and Cytospora canker of peach included timing as an experimental factor (19,26). In most studies, it is not known if efficacy was increased due to plant response (low susceptibility to infection), pathogen traits (low inoculum activity), or a combination of factors. Only the Phomopsis canker study on peach coordinated pruning time with annual spore production patterns (28). However, if the incubation period length of Phomopsis twig blight, which is not known, had been considered, an even greater disease reduction may have resulted. For those twig blight diseases in which pruning did not result in disease reduction, it is difficult to determine if the diseases possessed traits described by Van der Plank (29) which negatively influence sanitation efficacy, or if the lack of response was due to insufficient consideration of influential disease characteristics affecting pruning efficacy.

Based upon the results in this study, it is evident that fall, spring and summer are more similar to one another than they are to winter in terms of survival times and symptom expression rates. Twigs infected in spring, summer, and fall will generally become necrotic within or shortly after the season infection occurred. Based upon 95% confidence intervals of median survival times for the CTB severe disease stage, twigs infected in spring and summer can be expected to become necrotic within approximately 3.5 to 4.5 weeks, twigs infected in fall will become necrotic within approximately 4.5 to 6 weeks, and twigs infected in winter will become necrotic in approximately 8.5 to 11 weeks. Thus, twigs infected in winter will likely develop symptoms over a more prolonged period of time that may extend into spring. It is possible that disease symptoms expressed in spring may be the result of winter infection, or both winter infection and spring infection resulting from natural leaf senesce.

Influential factors may be quite similar between the initial onset of CTB and progression beyond the incubation period. However, results from the survival model should be interpreted cautiously since the temperature and season variables are inherently confounded. We cautiously conclude that temperatures between 15 and 30°C and seasonal effects have similar magnitudes of influence on CTB mild, moderate, and severe disease stages. The season variable represents more than just temperature since its definition was based upon host growth characteristics. Additionally, inclusion of the season variable improved model fit and colinearity was not an issue between the season and temperature variables. Hence, seasonal effects on CTB progression bear further study to determine exactly which parameters (climatic or otherwise) most affect symptom development.

Because smaller diameter twigs are at an increased risk for symptom development compared with larger diameter twigs, removal of smaller diameter twigs may prove to be a more efficacious control method. Canker formation on larger diameter twigs can result from progression of the pathogen from blighted smaller diameter twigs, and presumably form as the result of plant defenses to wall of the advancing pathogen (7). Cankers can exist for years without the stem progressing to a blighted stage. The perennial existence of the pathogen in cankers and the potential for cankers to serve as an inoculum source is unknown and bears further study.

Recurrent event modeling of CTB progression provides information about developmental expression from initial symptoms to complete distal twig blight that would be easily recognized and typically used as criteria for removal. The seasonal risks (hazard ratios) and relative rates (median ratios) of symptom development presented in this study provide information that can be used in a deterministic approach to CTB control. In fact, in viral infections of bunchy top disease of bananas, seasonal incubation period length has been used to model the appearance of symptoms and has been related to detection efficiency based upon the proportion of plants with asymptomatic, latent infections (1). The development of a more comprehensive, integrated disease management program for CTB will depend upon knowledge concerning the expression of symptoms, the number of sources or types of infected organs from which inoculum can be produced, and the synchronous nature of the inoculum production and infection processes.

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